Fig. 1A

GAAGCAAGGAGGCGGCGGCGGCCGAGCGAGTGGCGAGTAGTGGAAACGTTGC
TTCTGAGGGGAGCCCAAGATGACCGGTTCTAACGAGTTCAAGCTGAACCAGCC
ACCCGAGGATGGCATCTCCTCCGTGAAGTTCAGCCCCAACACCTCCCAGTTCCT
GCTTGTCTCCTCCTGGGACACGTCCGTGCGTCTCTACGATGTGCCGGCCAACTCC
ATGCGGCTCAAGTACCAGCACACCGGCGCCGTCCTGGACTGCGCCTTCTACGAT
CCAACGCATGCCTGGAGTGGAGGACTAGATCATCAATTGAAAATGCATGATT
TGAACACTGATCAAGAAAATCTTGTTGGGACCCATGATGCCCCTATCAGATGT
GTTGAATACTGTCCAGAAGTGAATGTGATGGTCACTGGAAGTTGGGATCAGA
CAGTTAAACTGTGGGATCCCAGAACTCCTTGTAATGCTGGGACCTTCTCTCAGC
CTGAAAAGGTATATACCCTCTCAGTGTCTGGAGACCGGCTGATTGTGGGAACA
GCAGGCCGCAGAGTGTTGGTGTGGGACTTACGGAACATGGGTTACGTGCAGCA
GCGCAGGGAGTCCAGCCTGAAATACCAGACTCGCTGCATACGAGCGTTTCCAA
ACAAGCAGGGTTATGTATTAAGCTCTATTGAAGGCCGAGTGGCAGTTGAGTA
TTTGGACCCAAGCCCTGAGGTACAGAAGAAGAAGTATGCCTTCAAATGTCAC
AGACTAAAAGAAAATAATATTGAGCAGATTTACCCAGTCAATGCCATTTCTT
TTCACAATATCCACAATACATTTGCCACAGGTGGTTCTGATGGCTTTGTAAAT
ATTTGGGATCCATTTAACAAAAAGCGACTGTGCCAATTCCATCGGTACCCCAC
GAGCATCGCATCACTTGCCTTCAGTAATGATGGGACTACGCTTGCAATAGCGT
CATCATATATGTATGAAATGGATGACACAGAACATCCTGAAGATGGTATCTT
CATTCGCCAAGTGACAGATGCAGAAACAAAACCCAAGTCACCATGTACTTGA
CAAGATTTCATTTACTTAAGTGCCATGTTGATGATAATAAAACAATTCGTAC
TCCCCAATGGTGGATTTATTACTATTAAAGAAACCAGGGAAAATATTAATTT
TAATATTATAACAACCTGAAAATAATGGAAAAGAGGTTTTTGAATTTTTTTTT
TTTAAATAAACACCTTCTTAAGTGCATGAGATGGTTTGATGGTTTGCTGCATT
AAAGGTATTTGGGCAAACAAAATTGGAGGGCAAGTGACTGCAGTTTTGAGA
ATCAGTTTTGACCTTGATGATTTTTTGTTTCCACTGTGGAAATAAATGTTTGTA
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TTAAACAAAGCGAGGTTAAGGTTAGACTCTTGGGAATCAGCTAGTTTTCAAT
CTTATTAGGGTGCAGAAGGAAAACTAATAAGAAAACCTCCTAATATCATTTT
GTGACTGTAAACAATTATTTATTAGCAAACAATTGATCCCAGAAGGGCAAAT
TGTTTGAGTCAGTAATGAGCTGAGAAAAGACAGAGCATATCTGTGTATTTGG
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CTCTGACATTTATATAGTTCTTATGTCCATTTCAGTTGACCAGCCGCTGGTGAT
TAAAGTTAAAAAGAAAAAAATTATAGTGAGAATGAGATTCATTTCAATGTA
ATGCACTAAAGCAGAACACGAACTTAGCTTGGCCTATTCTAGGTAGTTCCAA
ATAGTATTTTTGTTGTCAAACTTTAAAATTTATATTAATTTGCAAATGTATGT
CTCTGAGTAGGACTTGGACCTTTCCTGAGATTTATTTTATCCGTGATGTATTTT
TTTTAATTCTTTTGATACAGAGAAGGGTCTTTTTTTTTTTTTAAGTATTTCAGTGA
AAACTTGGTGTAAGTCTGAACCCATCTTTTGAAATGTATTTTCTTCATTGCAG

Fig. 1B

```
GTCCACCTAATCATCCTGTGAAAGTGGTTTCTCTATGGAAAGCTTTGTTTGCTT
CCTACAAATACATGCTTATTCCTTAAGGGATGTGTTAGAGTTACTGTGGATTT
CTCTGTTTTCTGTCTTACAAGAAACTTGTCTATGTACCTTAATACTTTGTTTAG
GATGAGGAGTCTTTGTGTGTCCCTGTACAGTAGTCTGACGTATTTCCCCTTCTGTC
CCCTAGTAAGCCCAGTTGCTGTATCTGAACAGTTTGAGCTCTTTTTGTAATATA
CTCTAAACCTGTTATTTCTGTGCTAATAAACGAGATGCAGAACCCTTGAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
```

Fig. 1B
(Continued)

Alignment of predicted huBUB3 gene products from ~1.4 Kb cDNA and ~2.7 Kb cDNA

```
                    20                      40                      60
huBUB3 2.7    MTGSNEFKLNQPPEDGISSVKFSPNTSQFLLVSSWDTSVRLYDVPANSMRLKYQHTGAVL
huBUB3 1.4    MTGSNEFKLNQPPEDGISSVKFSPNTSQFLLVSSWDTSVRLYDVPANSMRLKYQHTGAVL

                    80                     100                     120
huBUB3 2.7    DCAFYDPTHAWSGGLDHQLKMHDLNTDQENLVGTHDAPIRCVEYCPEVNVMVTGSWDQTV
huBUB3 1.4    DCAFYDPTHAWSGGLDHQLKMHDLNTDQENLVGTHDAPIRCVEYCPEVNVMVTGSWDQTV

                   140                     160                     180
huBUB3 2.7    KLWDPRTPCNAGTFSQPEKVYTLSVSGDRLIVGTAGRRVLVWDLRNMGYVQQRRESSLKY
huBUB3 1.4    KLWDPRTPCNAGTFSQPEKVYTLSVSGDRLIVGTAGRRVLVWDLRNMGYVQQRRESSLKY

                   200                     220                     240
huBUB3 2.7    QTRCIRAFPNKQGYVLSSIEGRVAVEYLDPSPEVQKKKYAFKCHRLKENNIEQIYPVNAI
huBUB3 1.4    QTRCIRAFPNKQGYVLSSIEGRVAVEYLDPSPEVQKKKYAFKCHRLKENNIEQIYPVNAI

                   260                     280                     300
huBUB3 2.7    SFHNIHNTFATGGSDGFVNIWDPFNKKRLCQFHRYPTSIASLAFSNDGTTLAIASSYMYE
huBUB3 1.4    SFHNIHNTFATGGSDGFVNIWDPFNKKRLCQFHRYPTSIASLAFSNDGTTLAIASSYMYE

                   320
huBUB3 2.7    MDDTEHPEDGIFIRQVTDAETKPKSPCT
huBUB3 1.4    MDDTEHPEDGIFIRQVTDAETKPKVHLIIL
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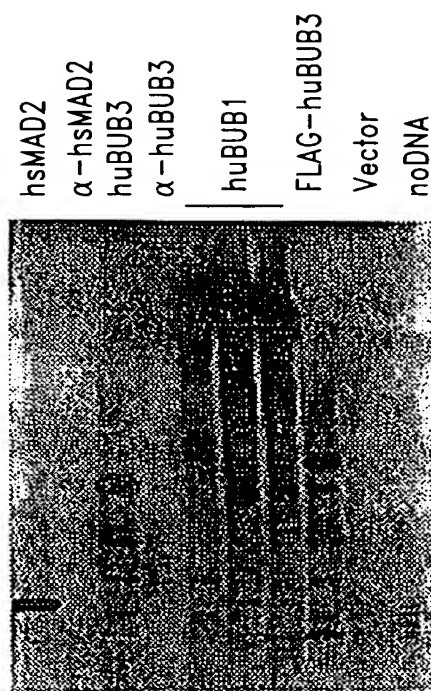
Fig. 2

```
scBUB3    1  ------------------------------MQIVQIEQAPKDYISDIKIIP---SKSLLL   27
muBUB3    1  ----------------------MTG-------SNEFKLNQPPEDGISSVKFSPN--TSQFLL   31
huBUB3    1  ----------------------MTG-------SNEFKLNQPPEDGISSVKFSPN--TSQFLL   31
rael-hu   1  MSLFGTTSGFGTSGTSMFGSATT--DNHNPMKDIEVTSSPDDSIGCLSFSPPTLPGNFLI   58
rael      1  MSLFG------QATTSTVSNATG-----DLKKDVEVAQPPEDSISDLAFSP---QAEYLA   46
yet7      1  MSFFNR-----SNTTSALGTSTAMANEKDLANDIVINSPAEDSISDIAFSP---QQDFMF   52
                                                    .    D I  . P

               ─────I─────                              ────II──
scBUB3   28  -ITSWDGSLTVYKFDIQAKNVDLLQSLRYKH--PLLCCNFI-DNTDLQIYVGTVQGEILK   83
muBUB3   32  -VSSWDT--SVRLYDVPA--NSM-R-LKYQHTGAVLDCAFY-DPT--HAWSGGLDHQLKM   81
huBUB3   32  -VSSWDT--SVRLYDVPA--NSM-R-LKYQHTGAVLDCAFY-DPT--HAWSGGLDHQLKM   81
rael-hu  59  -AGSWAN--DVRCWEVQDSGQTIPK-AQQMHTGPVLDVCWSDDGS--KVFTASCDKTAKM  112
rael     47  -ASSWDS--KVRIYEVQATGQSIGK-ALYEHQGPVLSVNWSRDGT--KVASGSVDKSAKV  100
yet7     53  SASSWDG--KVRIWDVQN-GVPQGR-AQHESSSPVLCTRWSNDGT--KVASGGCDNALKL  106
                SW      V    ..          .        .L      D  . .

             ─
scBUB3   84  VDLIGSPSFQALTNNEANLGICRICKYGD--DKLIAASWDGLIEVIDPRNYGDGVIAVKN  141
muBUB3   82  HDLNTDQENLVGTHDAPIRCVEYCPEVNV----MVTGSWDQTVKLWDPR-TPCNAGTFS-  135
huBUB3   82  HDLNTDQENLVGTHDAPIRCVEYCPEVNV----MVTGSWDQTVKLWDPR-TPCNAGTFS-  135
rael-hu 113  WDLSSNQAIQIAQHDAPVKTIHWIKAPNY--SCVMTGSWDKTLKFWDTR-SSNPMMVLQ-  168
rael    101  FDIQTGQNQQVAAHDDAVRCVRFVEAMGT-SPILATGSWDKTLKYWDLR-QSTPIATVS-  157
yet7    107  YDIASGQTQQIGMHSAPIKVLRFVQCGPSNTECIVTGSWDKTIKYWDMR-QPQPVSTVM-  164
             D.          .         .     . . SWD  .   D R
```

───III───

```
                ────IV───
scBUB3  142  LNSNNTKVKNKIFTMDTNSSRLIVGMNNSQVQWFRLPLCEDDNGTIEESGLKYQIRDVAL  201
muBUB3  136  Q-------PEKVYTLSVSGDRLIVGTAGRRVLVWDLWNMGYVQQRRE-SSLKYQTRCIRA  187
huBUB3  136  Q-------PEKVYTLSVSGDRLIVGTAGRRVLVWDLRNMGYVQQRRE-SSLKYQTRCIRA  187
rael-hu 169  L-------PERCYCADVIYPMAVVATAERGLIVYQLENQPSEFRRIE-SPLKHQHRCVAI  220
rael    158  L-------PERVYAMDCVHPLLTVATAERNICVINLSEPTKIFKLAM-SPLKFQTRSLAC  209
yet7    165  M-------PERVYSMDNKQSLLVVATAERHIAIINLANPTTIFKATT-SPLKWQTRCVAC  216
                .. .          V    . L           S LK Q R .
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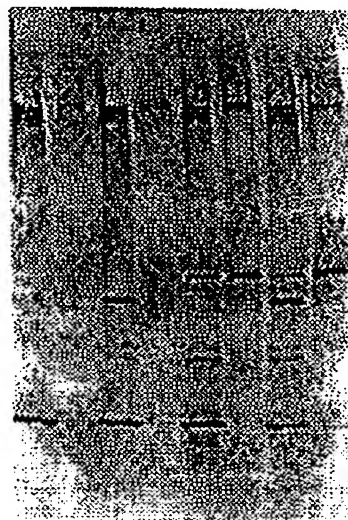
Fig. 3A

```
                           ——— V ———
scBUB3    202  LP---KEQEGYACSSIDGRVAVEFFDDQGDDYNSSKRFAFRCHRLNLKD-TNL-------  250
muBUB3    188  FP---NK-QGYVLSSIEGRVAVEYLDPS-PEV-QKKKYAFKCHRLKENN-IEQ-------  233
huBUB3    188  FP---NK-QGYVLSSIEGRVAVEYLDPS-PEV-QKKKYAFKCHRLKENN-IEQ-------  233
rael-hu   221  FKDKQNKPTGFALGSIEGRVAIHYINPP-NP-AKDNFTFKCHRSNGTNTSAP-----QD  272
rael      210  F----IKGDGYAIGSVEGRCAIQNIDE--KN--ASQNFSFRCHRNQAGN-SAD-------  253
yet7      217  Y----NEADGYAIGSVEGRCSIRYIDDG-MQ--KKSGFSFKCHRQTNPN-RAPGSNGQSL  268
                         G.    S..GR ..            ..F.CHR

                           ——— VI ———
scBUB3    251  AYPVNSIEFSPRHKFLYTAGSDGIISCWNLQTRKKIKNFAKFNED-SVVKIACSDNILCL  309
muBUB3    234  IYPVNAISFHNIHNTFATGGSDGFVNIWDPFNKKRLCQFHRYPTSIASLAFSNDGTTLAI  293
huBUB3    234  IYPVNAISFHNIHNTFATGGSDGFVNIWDPFNKKRLCQFHRYPTSIASLAFSNDGTTLAI  293
rael-hu   273  IYAVNGIAFHPVHGTLATVGSDGRFSFWDKDARTKLKTSEQLDQPISACCFNHNGNIFAY  332
rael      254  VYSVNSIAFHPQYGTFSTAGSDGTFSFWDKDSHQRLKSYPNVGGTISCSTFNRTGDIFAY  313
yet7      269  VYPVNSIAFHPLYGTFVTAGGDGTFNFWDKNQRHRLKGYPTLQASIPVCSFNRNGSVFAY  328
                    Y VN I F       T G DG    W      . . .                 .

scBUB3    310  ATSDDTFKTNAAIDQTIELNASSIYIIFDYEN--------  341
muBUB3    294  ASSYMYEMDDT-EHPE---DGIFIRQVTDAETKPKS---T  326
huBUB3    294  ASSYMYEMDDT-EHPE---DGIFIRQVTDAETKPKSPC-T  328
rael-hu   333  ASSYDWSKGHEFYNPQKK-NYIFLRNA-AEELKPRNKK--  368
rael      314  AISYDWSKGYTFNNAQLP-NKIMLHPVPQDEIKPRPKKGR  352
yet7      329  ALSYDWHQGHMGNRPDYP-NVIRLHATTDEEVKEK-KK-R  365
                    A S                    .          E
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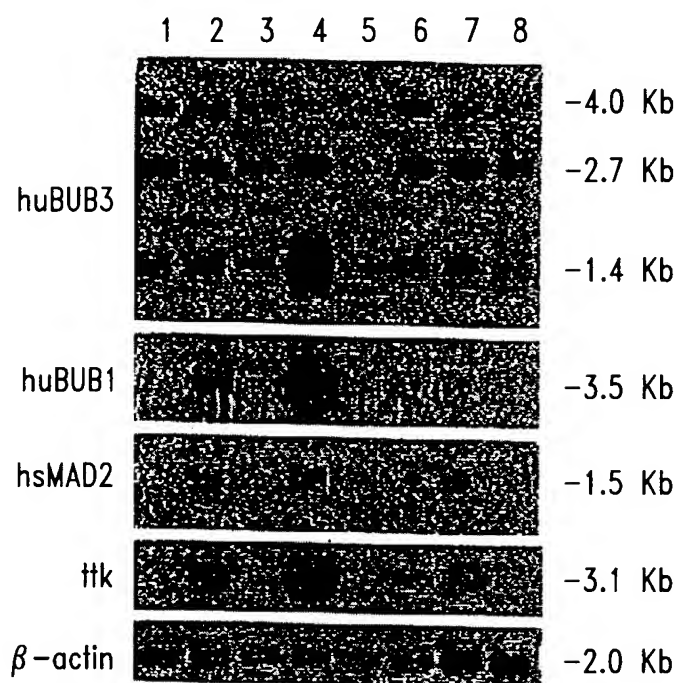
Fig. 3B

Fig. 4A

FLAG-huBUB3	−	−	+	+
huBUB3	−	+	−	+
huBUB1	+	+	+	+
hsMAD2	+	+	+	+
α-FLAG	S P	S P	S P	S P

Fig. 4B

huBUB1 (~121Kd)

FLAG-huBUB3
huBUB3 (~37Kd)

hsMAD2 (23Kd)

mRNA expression of BUB/MAD homologs in various tissues.

Fig. 5